

FRACTIONATION OF THE REACTION MIXTURE THROUGH A CARBON/CELITE COLUMN USING DIFFERENT SOLVENTS

In order to find the appropriate solvent system for the elution of the carbon/celite column, a typical reaction mixture was chromatographed using different water-alcohol mixtures as eluent. The results indicated that the volume of solvent was significantly reduced when a mixture of water:isopropanol was used as eluent.

Reaction conditions and results of fractionation:**a) Reaction (following the described procedure):**

- Nitrophenyl galactopyranoside, 5g.
- Xylose, 25 g.
- β -galactosidase from *E. Coli*.
- Phosphate buffer (pH, 7.0), 330 mL.
- 37 °C.

b) Fractionation through carbon/celite column:

Once the reaction is finished, the crude mixture is fractionated through a carbon/celite column using mixtures of alcohols and water as the eluent. The results are summarized in the following Table.

Table

Eluent	Eluent gradient (ratio water:alcohol)	Total volume (litres) used
water:methanol	100:0 (initial) \rightarrow 70:30(final)	12.9
water:ethanol	100:0 (initial) \rightarrow 90:10 (final)	7.5
water:isopropanol	100:0 (initial) \rightarrow 92:8(final)	5.8
water:isopropanol	98:2 (initial) \rightarrow 95:5(final)	3.6